K66 The Stability of Antidepressants and Antipsychotics in Dried Blood Spots (DBS) in Postmortem Cases

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Learning Overview: After attending this presentation, attendees will understand the reliability of the analysis of blood collected in postmortem cases and dried on a paper substrate (i.e., DBS), particularly for the detection and quantification of antidepressants and antipsychotics.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing new information about the use of DBS in forensic toxicology and the possibility of employing this technique as a good, simple, reliable, and complementary method of sample storage during autopsy.

Background and Goals: The analysis of blood collected and dried on a paper substrate (DBS) represents an alternative blood sample collection that is currently routinely used in neonatal screening. The analysis of DBS provides several advantages: (1) it requires a small volume of blood; (2) it needs easier sample handling and simple storage for a longer time periods at room temperatures; and (3) the dried blood matrix could limit or even avoid the postmortem changes, such as putrefaction or autolysis. The goals of this study were: (1) to develop a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for the determination of 22 antidepressants and 19 antipsychotics in DBSs; (2) to establish the diagnostic reliability of DBS with the routine blood analyses of these substances; and (3) to prove the stability of these analytes on DBS within a three-month period of storage.

Method: Aliquots of blood were pipetted on a five-spot filter paper (85µL of blood for each spot) in triplicate (so that further testing is possible). The cards were allowed to dry overnight in the dark at room temperature. For each analysis, a whole bloodstain was cut out and placed in a plastic tube containing 1mL phosphate buffer at pH 6 and deuterated internal standards, extracted using Solid Phase Extraction (SPE) cartridges, and injected into the LC/MS/MS system. The analytes were separated through a reverse phase chromatography on a C18 column and detected on a triple quad operating in Multiple Reaction Monitoring mode and positive polarization. A calibration curve was prepared in the range of Limit of Quantitation (LOQ) 500ng/mL.

Validation parameters (linearity, selectivity, specificity, accuracy, imprecision, recovery, matrix effects, and carry over) were tested. The procedure was applied to 60 authentic postmortem cases. For each case, 15 DBS were collected at T0. Four DBS were analyzed in triplicate within four consecutive weeks. The last samples were analyzed after three months.

Results: The analytical procedure is simple, sensitive, and specific. Limits of Detection (LODs) were detected in a range of 0.1ng/mL(g) to 4.1ng/mL(g) for antidepressants, and 0.1ng/mL to 2.7ng/mL for antipsychotics. LOQs varied from 0.3ng/ml to 13.6ng/ml for antidepressants and 0.3ng/ml to 9.1ng/mL for antipsychotics. Validation parameters fulfilled all the acceptance criteria. Eighteen different molecules were detected and quantitated in 22 out of 60 cases.

Four cases were positive for citalopram (42ng/ml–408ng/mL) and quetiapine (84 ng/ml –2309ng/mL), 3 for desvenlafaxine (479ng/ml–686ng/mL) and venlafaxine (25ng/ml–362ng/mL), 2 for paliperidone (17ng/ml–56ng/mL) and trazodone (91ng/ml–162ng/mL), while amisulpride (527ng/mL), clotiapine (25ng/mL), dibenzepine (483ng/mL), dixyrazine (126ng/mL), fluoxetine (56ng/mL), fluvoxamine (4,300ng/mL), haloperidol (20ng/mL), maprotiline (340ng/mL), mirtazapine (134ng/mL), and N-desmethylmirtazapine (112ng/mL), paroxetine (671ng/mL) and pimozide (130ng/mL) were found only once.

The concentrations on DBS stored at room temperature were in good agreement with the ones obtained on blood samples analyzed with routine methods (<30% CV, both at T0 and after three months).

The degradation percentage for most of the substances was lower than 20% within the three-month period. Citalopram, trimipramine, and paliperidone were stable for the first month, while a degradation higher than 50% was observed after three-month storage. Quetiapine was found to be stable into two samples, while a significant degradation was noticed in two other cases.

Conclusions: A method for the detection and quantitation of antidepressants and antipsychotics in DBS was successfully developed and validated. Preliminary results on 22 authentic positive postmortem cases suggested that DBS can be used as routine sample storage during autopsy. More cases will be analyzed to support the preliminary outcomes of this study.

Dried Blood Spot, Antidepressants, Antipsychotics